High-performance Liquid Chromatographic Determination of Organic Substances by Metal Chelate Derivatization. I. Dithiocarbamate Chelates of Aliphatic Amines

Masataka Moriyasu,* Yohei Hashimoto, and Masaru Endo**

Kobe Women's College of Pharmacy, Motoyamakita-machi, Higashinada-ku, Kobe 658

***Kinki District Narcotic Control Office, Uchikyuhoji-machi, Higashi-ku, Osaka 540*

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The use of the conversion of aliphatic primary and secondary amines into metal dithiocarbamate chelates was examined for high-performance liquid chromatographic determination of these amines. Characteristic chromatogram patterns based on the difference in the rate of ligand exchange were obtained for different central metal ions. When Hg(II) chelates were tested, trace determination of individual secondary amines was possible because only the peaks of binary complexes corresponding to each amine appeared on chromatograms. When Ni(II) and Pd(II) chelates were tested, peaks to ternary complexes as well as those of binary complexes appeared on chromatograms. This phenomenon was applied to the determination of optical purity of optically active amines. A description is given of applications to microdetermination of antiasthmatic ephedrine isomers, to determination of their optical purity in Chinese crude drugs, and to their preparation.

The metal chelate formation is widely applied to the colorimetric determination of metal ions. Since most chelating reagents are synthesized from organic substances, there have been made several attempts to determine organic substances after their conversion into metal chelates. Along with the recent progress of high-performance liquid chromatography(HPLC), a large number of UV-visible derivatization methods have been presented, but with little attention paid to utilization of the metal chelate formation for derivatization reactions of organic substances; this situation seems ascribable to the presumption that, as is often the case in gas chromatography, metal chelates usually undergo decomposition in course of chromatography. The purpose of the present series research is to clarify to what extent the metal chelate formation is useful for the UV-visible derivatization method in HPLC. The present report is concerned with conversion of aliphatic primary and secondary amines into dithiocarbamate chelates. We have described[1] an HPLC determination of various metal ions with the aid of their conversion into diethyldithiocarbamate chelates, and successively characterized[2,3] their chromatograms by means of ligand exchange. In experimental investigation on the application of the metal chelate formation to an analysis of organic substances, we should take into account the following two factors: (1) the organic substance needs to be converted into so stable a chelate as to undergo no decomposition in course of chromatography; (2) the ratio of central metal ion to ligand is of importance. If only 1:1 complexes are allowed to form, the determination of each organic substance may be performed easily. If 1:2 complexes are allowed to form, the ternary complex formation needs to be taken into consideration. When the ternary complex is very labile, it will undergo disproportionation into two binary complexes ($2MAB \rightarrow MA_2 + MB_2$) as soon as it gets separated; in this case, only the peaks for the binary complexes appear on the chromatogram, making the determination of individual organic substances possible. When the ternary complex is fairly inert, the peaks for ternary complex MAB will also appear on the chromatogram, complicating the chromatogram.

Experimental

Reagents. Sodium salts of various *N,N*-disubstituted and *N*-monosubstituted dithiocarbamic acids were prepared by mixing an aliphatic amine, carbon disulfide, and sodium hydroxide. The following aliphatic primary and secondary amines were used: dimethylamine, diethylamine, dipropylamine, dibutylamine, diisopropylamine, diisobutylamine, pyrrolidine, piperidine, perhydroazepine, morpholine, dibenzylamine, methylamine, ethylamine, propylamine, phenethylamine, and *l*-ephedrine. Each sodium salt was recrystallized from chloroform–methanol or chloroform–hexane. Standard solutions of each metal ion (0.1 M, 1 M=1 mol dm^{-3}) were prepared by dissolving each metal salt in water. The metal ions used are Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Ag(I), Cd(II), Hg(II), Pb(II), Bi(III), and Pd(II).

Apparatus. The HPLC apparatus used was the same as the one used previously.[1-3] Absorption spectra of metal chelates were recorded on a Model-124 Hitachi double-beam spectrometer.

Results and Discussion

Relationship between Stability Constant and Chromatographic Behavior of Metal Chelates. Solutions of various dithiocarbamate chelates were prepared by mixing each of sodium dithiocarbamates and each metal ion at a suitable pH. The chelate formed was extracted with chloroform. Except Fe(III) and Mn(III), all the dithiocarbamate chelates derived from the secondary amines gave solutions stable at least for 2 d. Since dithiocarbamates derived from primary amines in general have a strong reducing property, the metal chelates for Ag(I), Hg(II), and Cu(II) decomposed readily, whereas those for Pd(II), Ni(II), and Co(III) were stable.

The HPLC of various metal diethyldithiocarbamate chelates has been described in several reports[4-8] and ours.[1] Chromatographic behaviors of various dithiocarbamate chelates were examined. Of the chelates tested, Hg(II), Pd(II), Cu(II), Ni(II), and Co(III) chelates gave good chromatograms without any occurrence of decomposition. All of these chelates, except chemically unstable Hg(II) and Cu-

(II) chelates derived from primary amines, gave linear calibration curves over the range from a few μg to a few ng. Chelates of other metals, Mn(III), Fe(III), Zn(II), Cd(II), Pb(II), and Bi(III), sometimes accompanied decomposition when sample amounts were small, giving nonlinear calibration curves. Ag(I) chelates were adsorbed on the silica-gel surface too strongly to be eluted out. The stability of metal dithiocarbamate chelates has been investigated by several workers.[9-13] For example, Bode and Tusche reported the following order of increasing stability: Mn(III) < Fe(III) < Zn(II) < Cd(II) < Co(III) < Pb(II) < Bi(III) < Ni(II) < Cu(II) < Ag(I) < Pd(II) < Hg(II). The present results suggest that no decomposition occurs when the metal chelate is very stable (Hg(II), Pd(II), Cu(II), and Ni(II)) or robust (Co(III)).

Formation of Dithiocarbamate Chelates and Exchange of Central Metal Ion. An examination was made in pursuit for the optimum conditions for the formation of metal dithiocarbamate chelates from aliphatic amines. Since, of various metal ions, Hg(II), Pd(II), Cu(II), Ni(II), and Co(III) had formed chelates stable enough for amine analysis, the examination was limited to these metal ions. The formation of dithiocarbamates and metal chelates is expressed by

$$R_1R_2NH + CS_2 + OH^- \rightleftharpoons R_1R_2NCSS^- + H_2O \quad (1)$$

and

$$nR_1R_2NCSS^- + M^{n+} \longrightarrow M(R_1R_2NCSS)_n. \quad (2)$$

Reaction 1 cannot go to completion in the forward direction because in acidic media the backward reaction is allowed to proceed. As the metal chelate formation proceeds and the chelate formed is caused to go into the the organic layer, free dithiocarbamate ion in aqueous solution gets removed from the aqueous layer, thereby leading the entire reaction to completion. Of various procedures tested, the following was capable of giving quantitative results: To 1 cm^3 of dilute amine solution were added 1 cm^3 of 0.1 M standard solution of each metal ion, 1 cm^3 of conc aqueous ammonia, and 5 cm^3 of chloroform containing 2% carbon disulfide. The mixed solution in a test tube equipped with a ground glass stopper was shaken vigorously for about 30 s to complete the reaction. The chloroform layer was washed with water three times. Pd(II), Ni(II) and Co(III) chelates allowed the reaction to proceed quantitatively (>98%) for both primary and secondary amines. Cu(II) gave quantitative results only for secondary amines since the Cu(II) chelates obtained from primary amines were chemically unstable. This procedure was inapplicable to Hg(II) chelates because Hg(II) salts are insoluble in conc aqueous ammonia. Hg(II) chelates of secondary amines were formed quantitatively by means of exchange of central metal ion as follows. To chloroform solution of corresponding Ni(II) dithiocarbamate chelates, which are brownish yellow, $HgCl_2$ aqueous solution was added and the mixture was shaken vigorously for a few seconds. Since stability constants of Hg(II) chelates are much larger than those of Ni(II) chelates, exchange of central metal ion occurred according to

$$[Ni(R_1R_2NCSS)_2]_{org} + [Hg(II)]_w \longrightarrow [Hg(R_1R_2NCSS)_2]_{org} + [Ni(II)]_w, \quad (3)$$

where org and w denote the organic and the aqueous phase, respectively. Hg(II) chelates allowing no absorption in the visible range, the brownish yellow color of chloroform layer was caused to disappear, indicating the completion of the exchange of central metal ion. Hg(II) chelates thus prepared gave good chromatograms without any occurrence of decomposition.

Chromatograms of Ni(II) and Pd(II) Complexes. Various dithiocarbamate chelates were prepared by the methods given above and their elution behaviors put into examination. When diethylamine was used, the following elution sequence for central metal ions was obtained in parallel with increase in retention time: Hg(II) < Cu(II) < Ni(II) < Pd(II) < Co(III). Similar results were obtained for diisopropylamine and piperidine. For Ni(II) chelates derived from various amines was obtained the increasing order of retention time as follows: dibutylamine < diisobutylamine < dipropylamine < dibenzylamine < diisopropylamine < perhydrazepine < diethylamine < piperidine < pyrrolidine < dimethylamine < propylamine < phenethylamine < morpholine < ethylamine < methylamine < *l*-ephedrine; the retention time was found to increase with increase in the carbon number of alkyl chains. This may be interpreted in terms of the solubility of metal chelates in eluents because the solubility increases with increase in the chain length of hydrophobic alkyl groups. The prolonged retention times of morpholine and *l*-ephedrine may be interpreted from the fact that oxygen atoms included in these amines will be adsorbed strongly on the silica-gel surface.

Chromatograms of mixtures of two different amines were examined, the results being exemplified in Fig. 1. Both Ni(II) (Fig. 1(a)) and Pd(II) (Fig. 1(b)) gave three peaks. This implies that a ternary complex is formed according to the following equation and that the ternary complex formed is inert enough to be eluted out without being subjected to disproportionation during the course of chromatographic run:[2,3]

$$MA_2 + MB_2 \rightleftharpoons 2MAB \quad (4)$$

with

$$K = [MAB]^2/[MA_2][MB_2]. \quad (5)$$

This phenomenon which results in complicate chromatogram patterns has led Liska *et al.*[14] to claim that it is difficult to determine amines by application of the conversion into dithiocarbamate chelates. However, as described previously,[15] the present authors think that this phenomenon has some analytical value for the determination of optical purity of optically active amines. When an racemic mixture of amines exists in solution, the following optically active chelates will be formed:

$$MD_2 + ML_2 \rightleftharpoons 2MDL, \quad (6)$$

where D and L represent derivatives derived from *d*- and *l*-isomers, respectively. Since MD_2 and ML_2 are enantiomers and their chemical properties except optical activity are identical, they cannot be separated

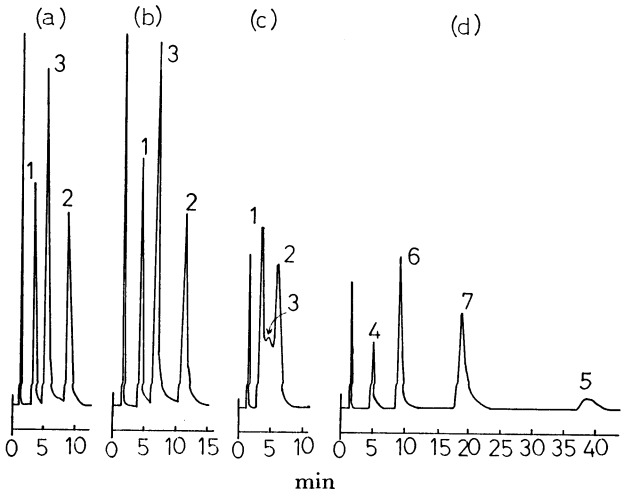

Fig. 1. Chromatogram patterns of dithiocarbamate chelates derived from the mixture of two amines.
Column: LiChrosorb SI 100 (4 mm×15 cm).
Eluent: hexane:ethyl acetate=100:3 (water saturated). Flow rate: 2.5 cm³/min. Detector: (a) 325 nm, (b) 308 nm, (c) 270 nm, (d) 268 nm. Sample: (a) Ni(II), (b) Pd(II), (c) Cu(II), (d) Co(III). Sample size: 50 μl (Concentrations of each amine were about 0.2 mM).
1: MA_2, 2: MB_2, 3: MAB, 4: MA_3, 5: MB_3, 6: MA_2B, 7: MAB_2. A=$(C_3H_7)_2NCSS^-$, B=$(CH_2)_5NCSS^-$.

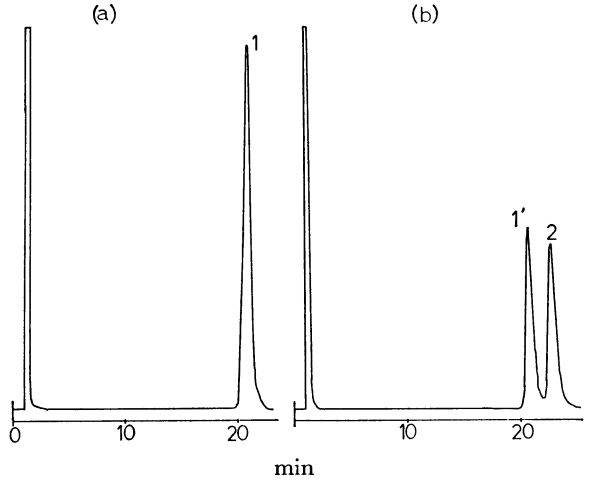

Fig. 2. Chromatograms of Ni(II) dithiocarbamate chelates derived from optically active and racemic ephedrines.
Column: LiChrosorb SI 100 (4 mm×25 cm).
Eluent: hexane:isopropyl acetate=100:11.5 (water saturated). Flow rate: 2.5 cm³/min. Detector: 325 nm. Sample: (a) 0.2 mM *l*-ephedrine(*d*-ephedrine gave similar chromatograms), (b) 0.2 mM *dl*-ephedrine Sample size: 50 μl.

from each other. On the other hand, MD_2 (or ML_2) and MDL are diastereomers, and can possibly be separated. Figures 2(a) and (b) show chromatograms of Ni(II) chelates derived from *l*- and *dl*-ephedrine, respectively. The *l*-isomer exists in nature and has widely been used as an antiasthmatic agent. On the contrary, *d*-isomer, not known to exist in nature, is ineffective as antiasthmatic agent. As shown in Fig. 2(b), the racemate gave two peaks, one being assigned to MD_2+ML_2 and the other to MDL. In Fig. 2(b), the peak area of the former (S_1) is equal to that of the latter (S_2), which suggests that the ternary complex formation according to Eq. 6 is controlled statistically ($K=[MDL]^2/([MD_2][ML_2])=4.0$). The optical purity of *dl*-mixture is determined as follows: When the initial concentrations of *d*- and *l*-isomers and d_0 and l_0, respectively, the equilibrium concentrations of MD_2 and ML_2 are $d_0-r/2$ and $l_0-r/2$, respectively, where r is the concentration of MDL. Since the ternary complex formation is controlled statistically, it is derived that $r=2d_0l_0/(d_0+l_0)$. The following relation will then hold between two peak areas S_1 and S_2:

$$\begin{aligned} S_2/(S_1+S_2) &= [MDL]/([MD_2]+[ML_2]+[MDL]) \\ &= r/(l_0+d_0) = 2d_0l_0/(d_0+l_0)^2 = 2p(1-p) \\ &= -2(p-1/2)^2+1/2, \end{aligned} \quad (7)$$

where $p=d_0/(d_0+l_0)$. Figure 3 shows parabolic plots for Eq. 7 with different ratios of *d*- to *l*-isomer. Both the calculated and observed results agreed well. The total ephedrine content was determined from the sum of the two peak areas S_1+S_2.

Pd(II) chelates gave similar results, with retention times slightly longer than those of corresponding Ni(II) complexes.

Chromatograms of Co(III) Complexes. It is well known that, when Co(II) ion reacts with dithiocarbamates to form metal chelates, Co(III) chelates are formed as a result of oxidation of Co(II) ion.[16] As shown in Fig. 1(d) four peaks, each assigned to MA_3, MA_2B, MAB_2, and MB_3, appeared on chromatograms when Co(II) solution was added. This result indicates that oxidation of the central metal ion occurs with formation of 1:3 complexes. No further investigation was made toward analytical application.

Chromatograms of Cu(II) Complexes. Figure 1(c) shows an example chromatogram for a mixture of two Cu(II) chelates. Since Cu(II) chelates are labile, their ternary complexes partially undergo disproportionation into two binary complexes during the course

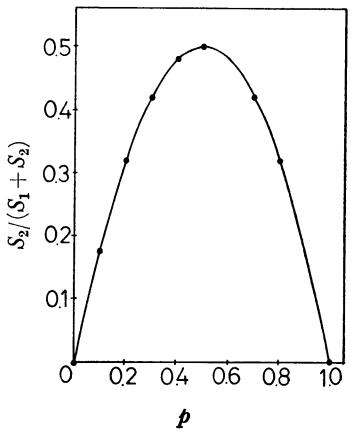

Fig. 3. Plot of Eq. 7 at various *d*- and *l*-isomer ratio chromatographic conditions were similar to those shown in Fig. 2.
——: Calculated value, ●: observed value.

of chromatographic run. The degree of the progress of disproportionation was sensitive to experimental conditions[3] such as the flow rate of pump and column temperature. The disproportionation within the column proceeded with increased proportions at higher column temperatures or at lower flow rates. Since the chromatogram pattern is very sensitive to experimental conditions, the analytical application seems unpromising.

Chromatograms of Hg(II) Complexes. Figure 4 shows chromatograms of Hg(II) chelates derived from mixtures of various aliphatic secondary amines. Unlike the other metal ions, only the peaks of the binary complexes corresponding to each amine appeared on chromatograms. The following aleternative explanations are plausible, the second being more likely: (1) no ternary complex formation occurs for Hg(II) chelates; (2) since Hg(II) complexes are very labile, the ternary complexes formed in the equilibrium state undergo disproportionation almost instantaneously after they are separated from the binary complexes. This seems a reasonable explanation because the rate of ligand exchange for Hg(II) complexes is known to be very high.

Figure 4 suggests that determination of individual amines will be possible when Hg(II) chelates are used. The mixture of samples shown in Fig. 4 was diluted to various concentrations and supplied for HPLC. The chromatograms were unsusceptible to experimental conditions and calibration curves were linear within a wide range of sample amounts. Thus, about 5 ng of each amine could be detected.

Application to Antiasthmatic Agents. Aliphatic amines usually show no or weak absorption in the UV or visible range. Since metal dithiocarbamate chelates show very strong absorption in th UV or visible range (log $\varepsilon > 4$), the present method will be useful for color-producing reactions. The present method was applied to the determination of ephedrines in crude drugs and Chinese crude drug preparations.

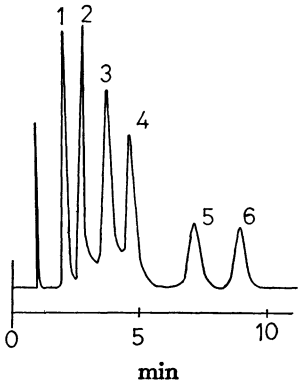

Fig. 4. Chromatograms of Hg(II) chelates derived from the mixture of various secondary amines.
Column: LiChrosorb SI 100 (4 mm×15 cm).
Eluent: hexane:ethyl acetate=100:3 (water saturated). Flow rate: 2.5 cm³/min. Detector: 273 nm. Sample size: 50 μl (Concentrations of each amine were about 0.03 mM).
1: Dipropylamine, 2: dibenzylamine, 3: diethylamine, 4: piperidine, 5: pyrrolidine, 6: dimethylamine.

Four ephedrine isomers, *l*-, *d*-, *l*-pseudo, and *d*-pseudoephedrine exist. Ephedra herba, which is one of the most important Chinese crude drugs and has widely been used as an antiasthmatic agent or for cough remedy, contains two isomers, *l*- and *d*-pseudoephedrine, the former being more effective. The synthesized racemates *dl*- and *dl*-pseudoephedrine are less pharmacologically active because *d*- or *l*-pseudoephedrine is not effective. Thus, the determination of total content and optical purity of ephedrines is important. Sample treatment was made as follows: to 200 mg of powdered ephedra herba or Chinese crude drug preparations, 40 cm³ of 0.5 M H_2SO_4 was added. The extraction was carried out for 1 hr three times. Then, to the combined extract 100 cm³ of 0.05 M $NiCl_2$ in conc aqueous ammonia and 40 cm³ of chloroform containing 2% carbon disulfide were added, and the mixture was shaken vigorously for about 30 s. A brownish yellow color appeared in the chloroform layer. The chloroform layer was washed with water three times and the

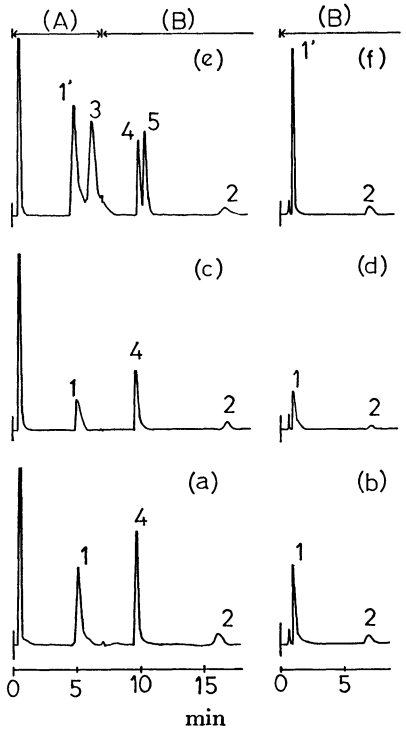

Fig. 5. Chromatograms of ephedrines in Ephedra herba and Chinese crude drug preparations.
Column: LiChrosorb SI 100 (4 mm×15 cm).
Eluent: hexane:isopropyl acetate=(A) 100:12 (B) 100:25 (water saturated). Flow rate: 2.5 cm³/min. Detector: (a)(c), and (e) 325 nm, (b), (d), and (f) 273 nm. Sample size: 50 μl.
Sample: (a) and (b) ephedra herba, (c) and (d) chinese crude drug preparations (Kakkonto), (e) and (f) ephedra herba+*d*-ephedrine: (a),(c), and (e) Ni(II) chelates (b), (d), and (f) Hg(II) chelates.
1: M(*l*-EP)$_2$, 1': M(*d*-EP)$_2$+M(*l*-EP)$_2$, 2: M(*d*-ϕ-EP)$_2$, 3: M(*l*-EP)(*d*-EP), 4: M(*l*-EP)(*d*-ϕ-EP), 5: M(*d*-EP)(*d*-ϕ-EP). *l*-EP, *d*-EP, and *d*-ϕ-EP denote dithiocarbamates derived from *l*-ephedrine, *d*-ephedrine, and *d*-ϕ-ephedrine, respectively.

extract was divided into two parts, one of which was supplied directly for HPLC (Ni(II) chelates). To the other part 1 mM $HgCl_2$ aqueous solution was added and the mixture was shaken for a few seconds. The brownish yellow color disappeared immediately as a result of the progress of the exchange of central metal ion according to Eq. 3. The solution was then supplied for HPLC (Hg(II) chelates). Figure 5 shows example chromatograms. Since *l*- and *d*-pseudoephedrine are diastereomers, two peaks corresponding to individual amines appeared on chromatograms when Hg(II) chelates were tested (Figs. 5(b) and (d)). Contrary to this, three peaks including the peak of ternary complex appeared on chromatograms when Ni(II) chelates were tested (Figs. 5(a) and (c)). Figures 5(a) and (c) show that no *d*-ephedrine is contained in these samples. Figure 5(e) shows chromatograms of Ni(II) chelates when a small amount of *d*-ephedrine was added. Two new peaks, assignable to ternary complexes M(*d*-EP)(*d*-ϕ-EP) and M(*d*-EP)(*l*-EP), appeared on chromatograms (Fig. 5(e)). The optical purity of *dl*-mixture of ephedrine in this sample can be determined by the following two methods: (1) equation 7 gives optical purity by comparing two peak areas of peaks 1′ ($M(l\text{-EP})_2+M(d\text{-EP})_2$) and 3 (M(*d*-EP)(*l*-EP)); (2) the ratio of two peak areas of peaks 4 (M(*l*-EP)(*d*-ϕ-EP)) and 5 (M(*d*-EP)(*d*-ϕ-EP)) directly indicates the ratio of *d*- and *l*-isomer. Contrary to the case with Ni(II) chelates, no chromatogram patterns of Hg(II) chelates were affected by addition of *d*-ephedrine (Fig. 5(f)). The peak height of the former was increased by addition of *d*-ephedrine while that of the latter unaffected. The area of the former peak 1 indicates the total content of *dl*-mixture of ephedrine. These results permit the calculation of individual contents of *l*-, *d*-, and *d*-pseudoephedrine.

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